Virulence 7:2, 70–71; February/March 2016; Published with license by Taylor & Francis Group, LLC

Hepatic inflammation resulting from localized, subcutaneous expression of Parvovirus VP1u capsid protein: Importance and implication

Laura A Adamson-Small[1] and Blanca Haydé Ruiz-Ordaz[2,*]

[1]Department of Pediatrics; Powell Gene Therapy Center; University of Florida; Gainesville, FL USA; [2]Department of Molecular Biology and Biotechnology; Biomedical Research Institute; National University of México; México City, Mexico

Keywords: Human parvovirus B19, inflammation, IVIS fluorescence, liver injury, parvovirus B19 VP1u Protein

This issue of *Virulence* features an article entitled "Human parvovirus B19 VP1u Protein as inflammatory mediators induces liver injury in naïve mice" which elucidated a relationship between expression of the Parvovirus B19 (B19) VP1-unique region (VP1u) and liver damage in a transgenic mouse model.[1] The *in vivo* effects of VP1u were investigated by subcutaneous injection of COS-7 cells transfected with an infrared reportable marker into Balb/c mice. Expression of the VP1u fusion protein was detectable up to 28 d following cell transfer by IVIS fluorescence imaging. A significant increase in inflammatory marker expression, STAT1 phosphorylation, and lymphocyte infiltration was observed in mice receiving COS-7 cells containing the VP1u expression plasmid. This study is the first to demonstrate a VP1u specific increase in liver inflammation from protein expression in non-hepatic cells.

Parvovirus B19 is a small, non-enveloped DNA virus whose main site of replication is in erythroid progenitor cells.[2] Transmission occurs through contaminated respiratory or blood droplets, but fetal transmission can also occur from infected mothers. Principal diseases associated with acute B19 infection include erythema infectiosum, transient aplastic crisis, and hydrops fetalis, but an association between B19 infection and other inflammatory or autoimmune diseases has been reported.[3-4] B19 produces 3 main proteins: a nonstructural protein, NS1, and 2 capsid proteins, VP1 and VP2, and 2 small proteins called the 7.5 and 11 kDa protein whose function has yet to be determined.[5] The capsid proteins are identical except for an N-terminus region on the VP1 protein called the VP1 unique (VP1u) region.[6]

In vitro, expression of B19 proteins has been shown to upregulate multiple inflammatory proteins including IL-6, TNF-α, and STAT3 phosphorylation.[7-10] Many of these studies have focused on NS1 expression, although VP1u has been shown to have phospholipase activity and can increase expression from the TNF-α and NF-κB promoters.[11-12] *In vivo*, a correlation between B19 infection and activations of inflammatory genes or pathways has also been reported both in intra-cellular expression and circulating cytokine detection.[12-14] Due to the strict specificity of B19 to human cells, there is no reliable animal model for wild type B19 infection to better explore these relationship between B19 infection and inflammatory disease.[15]

Current animal models to study the impact of B19 on disease including transgenic expression of B19 proteins, *ex vivo* transfection or transduction of cells injected into animals, or direct injection of B19 proteins or antibodies. Other animal models from this group have shown a correlation between B19 proteins or antibodies and liver inflammation. In one study, NZB/W F1 mice, a model of human systemic lupus erythematosus, were injected intravenously with an anti-VP1u antibody.[16] Liver tissue from these mice showed an increase in matrix metalloproteinase-9 activity and increase expression in the PI3K and ERK pathways. In a similar study, injection of VP1u antibodies were also shown to result in increased inflammation and lymphocyte infiltration in cardiac tissue, further demonstrating the potential immune impact of B19 VP1u antibodies.[17] In a subsequent study, B19 NS1, VP1u, VP2 were injected subcutaneously in NZB/W F1 mice.[18] Increased fibrosis and collagen deposition was observed in mice injected with the NS1 protein, but no marked changes were observed in those receiving the VP1u or VP2 protein. These data indicate that the results seen in the current study are a result of cellular expression of the VP1u protein and a potentially immune response against the viral protein.

Analysis of circulating cytokine profiles of human patients acutely infected with B19 and at 2-37 months following initial infection has demonstrated prolonged upregulation of inflammatory cytokines IFN-γ and TNF-α.[19] This increase was

*Correspondence to: Blanca Haydé Ruiz-Ordaz; Email: bhro@unam.mx
Submitted: 01/06/2016; Accepted: 01/06/2016
http://dx.doi.org/10.1080/21505594.2016.1141163

Comment on: Hsu, T-C et al. Human parvovirus B19 VP1u Protein as inflammatory mediators induces liver injury in naïve mice. http://dx.doi.org/10.1080/21505594

also associated with development of chronic fatigue. Interestingly, in this work, Hsu et al.[1] also reported an increase in IFN- γ in liver cells from mice injected subcutaneously with COS-7 cells expressing B19 VP1u protein. These data suggests B19 protein expression can elicit an indirect effect on other non-infected tissues. Further analysis of liver tissues from the VP1u expressing mice also demonstrated a significant increase in other inflammatory cytokines including MMP9/MMP2 ratios, CRP, IL-1β and IL-6 protein levels, and a marked increase in infiltrating lymphocytes, which may participate in liver injury, suggesting that B19-VP1u may have a role as mediators of inflammation during B19 infection. To determine the mechanism for this change in cytokine expression, changes in common inflammatory signaling pathways were examined. The authors also saw a significant increase in IKKα, IκB, phosphorylated P65, and phosphorylated STAT1 in liver tissues. Together, these data demonstrate that B19 VP1u protein expression can impact gene regulation in tissues not directly infected with B19 and this in turn can contribute to hepatic injury.

Research such as those reviewed here are beginning to evaluate the systemic effects of B19 infection and expression of viral proteins. While many previous studies have focused on the effects of the NS1 protein due to its cytotoxic effect on cells and ability to upregulate multiple inflammatory molecules, there is emerging evidence that the B19 VP1u may also play an important role in disease development through direct infection of cells or antibody formation.[12,21-22] Future studies examining virus inflammatory and antibody responses following VP1u expression may expand upon these findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Hsu TC, Chiu CC, Chang SC, Chan HC, Shi YF, Chen TY, Tzang BS. Human parvovirus B19 VP1u Protein as inflammatory mediators induces liver injury in naïve mice. Virulence 2015; 7(2); PMID:26632342; http://dx.doi.org/10.1080/21505594
2. Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004; 350:586-596; PMID:14762186; http://dx.doi.org/10.1056/NEJMra030840
3. Lehmann HW, von Landenberg P, Modrow S. Parvovirus B19 Infection and autoimmune disease. Autoimmunity Review 2003; 2(4): 218-223; http://dx.doi.org/10.1016/S1568-9972(03)00014-4
4. Adamson-Small LA, Ignatovich IV, Laemmerhirt MG, Hobbs JA. Persistent parvovirus B19 infection in non-erythroid tissues: possible role in the inflammatory and disease process. Virus Res 2014; 190:8-16; PMID:24998884; http://dx.doi.org/10.1016/j.virusres.2014.06.017
5. Berns KI, Bergoin M, Bloom M, Lederman M, Muzyczka N, Siegl G, Tal J, Tattersall P. Family Parvoviridae. In: Regenmortel MHV, Fauquet CM, Bishop DHL, et al., editors. Virus taxonomy: classification and nomenclature of viruses: seventh report of the International Committee on Taxonomy of Viruses. New York: Academic Press; 2000. p. 169-78.
6. Agbandje M, Kajigaya S, McKenna R, Young NS, Rossmann MG. The structure of human parvovirus B19 at 8 Å resolution. Virology 1994; 203:106-15.; PMID:8030266; http://dx.doi.org/10.1006/viro.1994.1460
7. Moffatt S, Tanaka N, Tada K, Nose M, Nakamura M, Muraoka O, Hirano T, Sugamura K. A cytotoxic nonstructural protein, NS1, of human parvovirus B19 induces activation of interleukin-6 gene expression. J Virol 1996; 70(12): 8485-91; PMID:8970971
8. Fu Y, Ishii KK, Munakata Y, Saitoh T, Kaku M, Sasaki T. Regulation of tumor necrosis factor alpha promoter by human parvovirus B19 NS1 through activation of AP-1 and AP-2. J Virol 2002; 76(11): 5395-403; PMID:11991968; http://dx.doi.org/10.1128/JVI.76.11.5395-5403.2002
9. Hsu TC, Tzang BS, Huang CN, Lee YJ, Liu GY, Chen MC, Tsay GJ. Increased expression and secretion of interleukin-6 in human parvovirus B19 non-structural protein (NS1) transfected COS-7 epithelial cells. Clin Exp Immunol 2006; 144(1): 152-7; PMID:16542377; http://dx.doi.org/10.1111/j.1365-2249.2006.03023.x
10. Duechting A, Tschöpe C, Kaiser H, Lamkemeyer T, Tanaka N, Aberle S, Lang F, Torresi J, Kandolf R, Bock CT. Human parvovirus B19 NS1 protein modulates inflammatory signaling by activation of STAT3/PIAS3 in human endothelial cells. J Virol 2008; 82 (16): 7942-52; PMID:18550668; http://dx.doi.org/10.1128/JVI.00891-08
11. Dorsch S, Liebisch G, Kaufmann B, von Landenberg P, Hoffmann JH, Drobnik W, Modrow S. The VP1 unique region of parvovirus B19 and its constituent phospholipase A2-like activity. J Virol 2002; 76(4): 2014-8.; PMID:11799199; http://dx.doi.org/10.1128/JVI.76.4.2014-2018.2002
12. Deng X, Dong Y, Yi Q, Huang Y, Zhao D, Yang Y, Tijssen P, Qiu J, Liu K, Li Y. The determinants for the enzyme activity of human parvovirus B19 phospholipase A2 (PLA2) and its influence on cultured cells. PLoS One 2013; 8(4): e61440
13. Takahashi Y, Murai C, Shibata S, Munakata Y, Ishii T, Ishii K, Saitoh T, Sawai T, Sugamura K, Sasaki T. Human parvovirus B19 as a causative agent for rheumatoid arthritis. PNAS 1998; 95(14): 8227-32; PMID:9653169; http://dx.doi.org/10.1073/pnas.95.14.8227
14. Isa A, Lundqvist A, Lindblom A, Tolfvenstam A, Broliden K. Cytokine responses in acute and persistent human parvovirus B19 infection. Clin Exp Immunol 2007; 147(3): 419-425; PMID:17302890; http://dx.doi.org/10.1111/j.1365-2249.2006.03286.x
15. Wang JH, Zhang WP, Liu HX, Wang D, Li YF, Wang WQ, Wang L, He FR, Wang Z, Yan QG, Chen LW, Huang GS. Detection of human parvovirus B19 in papillary thyroid carcinoma. Br J Cancer 2008; 98(3): 611-8; PMID:18212749; http://dx.doi.org/10.1038/sj.bjc.6604196
16. Heegaard ED, Brown KE. Human Parvovirus B19. Clin Microbiol Rev 2002; 15(3): 485-505; PMID:12097253; http://dx.doi.org/10.1128/CMR.15.3.485-505.2002
17. Tsai CC1, Tzang BS, Chiang SY, Hsu GJ, Hsu TC. Increased expression of Matrix Metalloproteinase 9 in liver from NZB/W F1 mice received antibody against human parvovirus B19 VP1 unique region protein. J Biomed Scie 2009; 16:14; http://dx.doi.org/10.1186/1423-0127-16-14
18. Tzang BS, Lin TM, Tsai CC, Hsu JD, Yang LC, Hsu TC. Increased cardiac injury in NZB/W F1 mice received antibody against human parvovirus B19 VP1 unique region protein. Mol Immunol 2001; 48(12-13):1518-24.
19. Hsu TC, Tsai CC, Chiu CC, Hsu JD, Tzang BS. Exacerbating Effects of Human Parvovirus B19 NS1 on Liver Fibrosis in NZB/W F1 Mice. PLoS ONE 2013; 9(1): 10.1371.
20. Kerr JR, Barah F, Mattey DL, Laing I, Hopkins SJ, Hutchinson IV, Tyrrell DA. Circulating tumour necrosis factor-alpha and interferon-gamma are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. J Gen Virol 2001; 82: 3011-9; PMID:11714978; http://dx.doi.org/10.1099/0022-1317-82-12-3011
21. Moffatt S, Tanaka N, Tada K, Nose M, Nakamura M, Muraoka O, Hirano T, Sugamura K. A cytotoxic nonstructural protein, NS1, of human parvovirus B19 induces activation of interleukin-6 gene expression. J Virol 1996; 70(12): 8485-91; PMID:8970971
22. Li Y, Wang J, Zhu G, Zhang X, Zhai H, Zhang W, Wang W, Huang G. Detection of parvovirus B19 nucleic acids and expression of viral VP1/VP2 antigen in human colon carcinoma. Am J Gastroenterol 2007; 102(7): 1489-98; PMID:17459020; http://dx.doi.org/10.1111/j.1572-0241.2007.01240.x